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A STUDY OF CHEMICAL AND CELLULAR CHANGES IN THE BLOOD

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A STUDY OF CHEMICAL AND CELLULAR CHANGES IN THE BLOOD^{1, 2, 3}

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The physiological changes resulting from prolonged residence in atmospheres deficient in oxygen have been studied for many years. The work reported in this paper is an approach to a similar study of the response to residence in atmospheres containing an excess of carbon dioxide.

METHODS. Normal unanesthetized dogs which were fed a standard diet were used in all the experiments. Acid-base and hematological standards were established for each dog during a preliminary control period. At the end of the control period, the dog was placed in a respiration chamber of 144 cubic feet capacity, in which temperature, humidity and carbon dioxide and oxygen concentrations were controlled. The carbon dioxide concentration in the chamber was slowly increased by accumulation of the dog's expired air until the desired level had been reached (ranging from 1.5 to 5.0 per cent in different experiments). This level was then controlled by means of an adjustable stream of compressed air passing through the chamber. Oxygen was delivered continuously from a 50 liter spirometer and the oxygen concentration was maintained at 21 per cent in one series of experiments and 30 to 35 per cent in another series. Analyses were performed at intervals on blood samples drawn anaerobically over mercury from the femoral artery. Gas-free isotonic 1.0 per cent heparin solution was used for the anticoagulant and all values were corrected for the dilution of the blood with heparin solution. The analytical methods used were as follows (all analyses were performed in duplicate, and the results were required to check within the generally accepted limits of error). Red and white cell counts were made with

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⁴ A few of the experiments upon which this paper is based were performed at the Wm. H. Maybury Sanatorium (Detroit Municipal Tuberculosis Sanatorium), Northville, Michigan.

pipettes and counting chambers certified by the Bureau of Standards. Hemoglobin was determined by the oxygen capacity method of Van Slyke and Neill (1924) with saturation in a Barcroft tonometer. Reticulocyte counts were made on moist preparations stained supra-vitally at 38°C. with brilliant cresyl blue. Hematocrit cell volume was determined by a method previously described (Miller, 1939). pH was determined at 38°C. with the MacIness glass electrode (Hill, 1931). Carbon dioxide and oxygen contents were estimated by the manometric method of Van Slyke and Neill (1924). Chloride determinations were made on whole blood and plasma by Whitehorn's modification of the Volhard titration (Whitehorn, 1921) and cell chloride was calculated from these values and the hematocrit cell volume. Plasma protein was estimated by the colorimetric method of Wu, as modified by Greenberg (1929). All the other values presented in this paper were calculated from the experimentally determined results by nomographic or equational methods.

RESULTS. Thirty-two experiments were performed, with periods of exposure to carbon dioxide ranging from 1 to 4 weeks. The results will be discussed under the three headings of acid-base balance, water and electrolyte equilibria and hematology.

Acid-base balance. The changes in acid-base balance are summarized in table 1. The condition obtaining in most of the experiments was that of a mild, uncompensated acidosis. The average arterial CO₂ tension increased from 38.1 to 51.7 mm. Hg. The highest CO₂ tension observed was 78.0 mm. The average pH decreased from 7.38 to 7.26 an increase of 32 per cent in the hydrogen ion concentration. In the majority of cases, the pH during hypercapnia fell within the limits 7.20 to 7.30. The lowest pH observed in any experiment was 7.12, and occasionally there was a secondary alkaline swing.

The changes in whole blood carbon dioxide content were complex. In 6 of 13 dogs, there was an initial average increase of 11.3 per cent (from a normal average of 40.14 vol. per cent to a hypercapnic average of 45.27 vol. per cent). In the 7 other dogs, whole blood carbon dioxide content decreased 12.9 per cent (from a normal average of 41.70 vol. per cent to a hypercapnic average of 36.31 vol. per cent). In two of the animals having an initial increase, there was a secondary decline, so that the final trend in 9 of 13 cases was a decrease in whole blood CO₂ content. This decrease was associated with a decline in average carbon dioxide capacity from 48.5 vol. per cent to 34.6 vol. per cent. The decline in carbon dioxide capacity is comparable in magnitude to that obtained by Gesell and co-workers (1930) when high carbon dioxide mixtures were administered to anesthetized dogs under constant artificial ventilation. In experiments on dogs, Shaw and Messer (1932) found that inhalation of 10 per cent carbon dioxide caused a decrease in the carbon dioxide com-

binning power of the blood. To explain this result, they proposed a theory based on the relative carbon dioxide combining power of blood and tissues. Since the buffer power of blood is higher than that of tissues, more bicarbonate will be formed in the blood than in the tissues (per unit volume) when the alveolar carbon dioxide tension is increased. Then, to restore the equilibrium distribution of bicarbonate between blood and tissues, there is a shift of bicarbonate from blood to tissues. This is somewhat the same idea previously postulated by Gesell and co-workers (loc. cit., 1930). They believed that the increase in blood lactic acid and decrease in carbon dioxide capacity resulting from the administration of high carbon dioxide mixtures (in the experiments referred to above) were

TABLE 1

Effect of hypercapnia on the acid-base balance of the blood in 13 dogs

	pH	PLASMA CO ₂ TENSION	PLASMA CO ₂ CAPACITY	W.B. CO ₂ CONTENT	HCO ₃ ⁻ *
		mm. Hg	vol. per cent	vol. per cent	
Normal average	7.38	38.1	48.5	See text	0.80
Hypercapnic average	7.26	51.7	34.6	See text	0.85
Per cent change		35.7	28.6		

* This was calculated from values of cell and plasma bicarbonate expressed in milli-equivalents per liter of cell or plasma water.

Respiration chamber contained 1.5 to 5.0 per cent CO₂ and 21 to 22 per cent O₂.

Period of exposure was 1 to 4 weeks.

TABLE 2

Effect of hypercapnia on blood cells and hemoglobin in 10 dogs

	ERYTHROCYTES	LEUCOCYTES	HEMOGLOBIN	M.C. HEMOGLOBIN	M.C. VOLUME	HEMOGLOBIN SATURATION
	mil- lions	thou- sands	grams per 100 cc.	gam- ma gam- ma	cu. micra	
Normal average	7.22	14.5	16.7	23.0	62.7	94.7
Hypercapnic average	8.08	26.5	18.4	22.8	64.6	91.1
Per cent change	11.8	86.3	10.2	0.9	3.0	3.6

Respiration chamber contained 1.5 to 5.0 per cent CO₂ and 21 to 22 per cent O₂.

Period of exposure was 1 to 4 weeks.

due to an exchange of blood bicarbonate for an equivalent amount of tissue lactate. In view of the findings of Adolph, Nance and Shiling (1929) that from 40 to 700 cc. of carbon dioxide are retained by the body for each increase of 1 mm. in the alveolar carbon dioxide tension, this buffer action of the tissues becomes of great significance. As an accessory buffer action of the tissues, Simpson and Wells (1928) reported a chloride shift from tissues to blood when dogs were made to breathe 50 per cent carbon dioxide. This result has not been verified in the present work (however, much lower carbon dioxide concentrations were used). Detailed studies of blood and tissue bicarbonate, lactate and chloride during chronic hypercapnia would be of interest.

Water and electrolyte equilibria. The shifts of water and electrolytes between cells and plasma in the present experiments were qualitatively

consistent with predictions from the Donnan theory and known osmotic relationships.

The changes in mean cell volume are presented in table 2. In 5 experiments there was an increase in mean cell volume, in 3 there was a decrease, and in 1 there was no change. In all three cases in which the mean cell volume decreased, the whole blood carbon dioxide content was likewise diminished, while in 1 of the 5 experiments in which the mean cell volume increased there was a small diminution in whole blood carbon dioxide content. Thus the mean cell volume varied in direct proportion (though not quantitatively) with the whole blood carbon dioxide content in 7 out of 9 cases. The lack of complete and quantitative agreement between fact and theory may be attributed to several factors, among which are the inaccuracy of the hematocrit method (Millar, 1925) and the possibility of compensatory shift of water from tissues to plasma to maintain the constancy of the plasma volume (Simpson and Wells, 1928). Eisenman, Bulger and Peters (1926) found that when the carbon dioxide tension of a sample of oxygenated blood was increased from 30 to 60 mm., there was an actual shrinkage of the red cells in 5 out of 19 cases. They attributed this result to the inaccuracy of the hematocrit method.

In 12 of 13 experiments, there was an increase in the ratio cell bicarbonate: plasma bicarbonate ($r_{\text{HCO}_3^-}$ increased from 0.80 to 0.85). In the single experiment in which r did not increase, there was a transient small increase followed by a return to normal. The increased ratio persisted during the secondary fall in whole blood carbon dioxide content, suggesting that the controlling factor may be pH and not carbon dioxide *per se*.

In 4 experiments, studies were made of the changes in chloride distribution between cells and plasma. In one experiment there was no significant change, while in the other three, there was a definite shift of chloride from plasma to cells, as indicated by the increase in r_{Cl^-} from 0.74 to 0.87. In two experiments there was a striking decrease in whole blood chloride, amounting to 14.9 and 18.2 per cent respectively. Evidence is not available as to whether this is to be interpreted as a transfer of chloride from blood to tissues or as a urinary excretion of chloride. Simpson (1929) found that acute administration of 10 per cent carbon dioxide to anesthetized dogs caused a decreased urinary excretion of chloride.

Hematological changes. In 1915, Dallwig, Kolls and Loevenhart reported an increase of 8 to 10 per cent in the erythrocyte count of dogs which had been exposed to 0.5 to 1.0 per cent carbon dioxide in a respiration chamber for a period of one week. They believed that the stimulation of the bone marrow was due to the acid effect of carbon dioxide in diminishing the supply of oxygen to the bone marrow. Dufton (1927) observed that the erythrocyte count of rabbits increased about 20 per

cent when they were exposed to 3 to 4 per cent carbon dioxide for 4 days. In neither of these studies was the number of experiments adequate, nor were control experiments performed to prove the bone marrow origin of the response. Accordingly, a more thorough investigation seemed desirable.

In 10 experiments, with 1.5 to 5.0 per cent carbon dioxide and 21 per cent oxygen, the erythrocyte count increased in all but one experiment (see table 2). The average increase was 11.8 per cent (from 7,220,000 to

TABLE 3

Effect of splenectomy on erythrocyte response to CO₂

DOG NUM- BER	CONDITION	ERYTHRO- CYTES		PER CENT CHANGE
		Normal	Hyper- capnia	
		mil- lions	mil- lions	
1	Normal	7.44	8.51	14.4
2	Splenec- tomized	7.07	8.12	14.8
3	Normal	7.26	8.31	14.5
4	Splenec- tomized	6.98	8.02	14.9

Dogs 1 and 2 were tested together during one exposure period and dogs 3 and 4 during a second exposure period.

Respiration chamber contained 1.5 to 2.5 per cent CO₂ and 21 to 22 per cent O₂.

Period of exposure was 14 days.

TABLE 4

Effect of oxygen excess on erythrocyte response to CO₂ in 3 dogs

	ERYTHROCYTES	RETICULOCYTES	HEMOGLOBIN	M.C. HEMOGLOBIN	HEMOGLOBIN SATURATION
	millions	per cent	grams per 100 cc.	gamma gamma	
Normal	7.22	0.5	17.0	23.5	94.0
Hypercap- nic aver- age	8.01	7.2	17.9	22.3	95.1
Per cent change	11.0	6.7	5.3	5.1	1.1
Per cent change in 21 per cent oxy- gen	11.8		10.2	0.9	3.6

Respiration chamber contained 2.5 to 5.0 per cent CO₂ and 30 to 35 per cent O₂.

Period of exposure was 3 weeks.

8,080,000), and was practically independent of the carbon dioxide concentration within the limits stated. The hemoglobin concentration increased somewhat irregularly, usually lagging behind the erythrocyte response, but the final maximum was of the same order of magnitude, representing an increase of 10.2 per cent (from 16.7 to 18.4 grams per 100 cc). The rise in erythrocyte count began after a variable latent period of 4 to 8 days and reached a maximum in about 3 weeks. In several of these experiments, numerous plasma protein determinations were made. The absence of any significant change, in spite of a well marked erythrocyte increase, was presumptive evidence against the occurrence of hemoconcentration. The

effect of splenectomy on the erythrocyte response to carbon dioxide is shown in table 3. The magnitude of the erythrocyte increase in the splenectomized animals was identical with that in the normal control animals.

A decrease in the oxygen saturation of the arterial blood in these experiments (from 94.7 to 91.1 per cent) suggested the possibility that arterial anoxia might play a significant rôle in the erythrocyte increase. Accordingly, 5 experiments were performed in which the oxygen concentration in the respiration chamber was maintained at 30 to 35 per cent, and the carbon dioxide concentration at 2.5 to 5.0 per cent. The results of 3 of these experiments are summarized in table 4. The erythrocyte increase is nearly identical with that observed in the first series of experiments, although the hemoglobin increase is only half as great (possibly due to dietary factors, since the dogs in this series were on a diet considerably lower in protein than were the dogs in the first series). The other two dogs of the second series (data not included in table 4) had low pre-test erythrocyte counts, of unknown origin, which had remained constant for some weeks prior to the experiments. The erythrocyte increase in these two animals was very large (54.4 and 59.6 per cent, respectively, with correspondingly high reticulocyte counts). Since the usual erythrocyte increase occurred in this second series, in spite of the fact that the percentage saturation of the arterial blood *increased* (from 94.0 to 95.1 per cent), it is apparent that *arterial* anoxia is not a contributing factor. The possibility of tissue anoxia will be discussed later.

The total leucocyte count increased in 13 out of 15 experiments. The average normal leucocyte count in the 15 dogs studied was 14,500 cells per cu. mm., with a range of 10,000 to 24,000, which is of the order of magnitude reported by Bruner and Wakerlin (1937). The count rose rapidly during the first two or three days in the respiration chamber, reaching a maximum in less than a week. In some cases this maximum was maintained for the duration of the experiment, while in others it declined to a level somewhat above normal. The average increase in 15 experiments was 86.3 per cent, with individual cases ranging from a decrease of 17.4 per cent to an increase of 215.0 per cent. There was no significant change in the differential count aside from a slight increase in the neutrophil-lymphocyte ratio. No satisfactory explanation has been found for this leucocytosis, and no reference to the effect of chronic hypercapnia on the leucocyte count has been found in the literature.

DISCUSSION. Evidence has steadily increased in recent years in support of the concept that, at least so far as respiration is concerned, the actions of carbon dioxide excess and oxygen lack may partially converge to the common ground of increased tissue acidity and depressed tissue oxidations (Gesell, 1925, 1929, 1939). If this be true, then the fundamental tissue reaction to oxygen lack and to carbon dioxide excess should

be similar. In other words, the adaptive process should work toward the same goal in each case.

The most striking adaptive change to chronic oxygen lack is the almost invariable increase in erythrocyte count and hemoglobin concentration. This phenomenon is known to be due to increased bone marrow activity, presumably the result of a tissue anoxia of the bone marrow itself. The present work has demonstrated that carbon dioxide also is capable of stimulating the bone marrow, even when the arterial oxygen tension and content are supernormal. It is postulated that this stimulation is the result of a tissue anoxia induced by the increased tissue acidity. The supply of oxygen to the bone marrow is adequate, but the oxidative mechanisms have been depressed by the acid effect of carbon dioxide. Gesell and co-workers (1930) found that the administration of high carbon dioxide mixtures to anesthetized dogs depressed tissue oxidations even in the presence of supernormal oxygen tensions. Whatever the mechanism by which the bone marrow is stimulated to increased activity during chronic hypercapnia, the adaptive significance is obvious. An increase in the amount of circulating hemoglobin furnishes the tissues with an increased amount of oxygen with which to combat the depression of tissue oxidations resulting from increased tissue acidity. It also provides an increased amount of the buffer which is responsible, directly or indirectly, for the transport of about 90 per cent of all the carbon dioxide carried in the blood.

The leucocytosis of chronic hypercapnia has no obvious adaptive significance, so far as respiration and acid-base balance are concerned. It does not occur in chronic oxygen lack (Schneider, 1921; Myer, SeEVERS and Beatty, 1935).

The decrease in whole blood chloride during hypercapnia, provided it is due to urinary excretion of chloride unaccompanied by fixed base, is of advantage in liberating extra base for buffering carbon dioxide. Evidence on this point is conflicting. Water and electrolyte transfers between blood plasma and erythrocytes during chronic hypercapnia are readily explained by relatively simple physico-chemical laws, and are of doubtful adaptive value.

SUMMARY

When dogs were exposed to atmospheres containing 1.5 to 5.0 per cent carbon dioxide for periods of 1 to 4 weeks, the following changes were observed:

1. There was a mild, uncompensated acidosis (pH 7.20 to 7.30) with a decline in carbon dioxide combining power. This is interpreted as an exchange of blood bicarbonate for tissue lactate or chloride.

2. There was a transfer of chloride from plasma to erythrocytes and a decrease in whole blood chloride.

3. The ratio of cell bicarbonate to plasma bicarbonate increased, as did the mean corpuscular volume of the erythrocytes.

4. Erythrocyte count and hemoglobin concentration increased after a variable latent period. The bone marrow origin of this increase was indicated by the rise in reticulocyte count, the negative influence of splenectomy and the absence of hemoconcentration. The administration of 35 per cent oxygen with the carbon dioxide, although increasing the saturation of the arterial blood above normal, had no effect on the erythrocyte increase. It is concluded that the bone marrow stimulation was due to a tissue anoxia resulting from depression of tissue oxidations by carbon dioxide.

5. The total leucocyte count increased greatly but there was no significant change in the differential count.

It gives me pleasure to acknowledge my indebtedness to Prof. Robert Gesell for his constant interest in this problem and for his constructive advice and criticism.

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